

WHAT IS CLAIMED IS:

1. An isolated or recombinant nucleic acid comprising a nucleic acid sequence having at least 50% sequence identity to SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:41, SEQ ID NO:43, SEQ ID NO:45, SEQ ID NO:47, SEQ ID NO:49, SEQ ID NO:51, SEQ ID NO:53, SEQ ID NO:55, SEQ ID NO:57, SEQ ID NO:59, SEQ ID NO:61, SEQ ID NO:63, SEQ ID NO:65, SEQ ID NO:67, SEQ ID NO:69, SEQ ID NO:71, SEQ ID NO:73, SEQ ID NO:75, SEQ ID NO:77, SEQ ID NO:79, SEQ ID NO:81, SEQ ID NO:83, SEQ ID NO:85, SEQ ID NO:87, SEQ ID NO:89, SEQ ID NO:91, SEQ ID NO:93, SEQ ID NO:95, SEQ ID NO:97, SEQ ID NO:99, SEQ ID NO:101, SEQ ID NO:103, SEQ ID NO:105, over a region of at least about 100 residues, wherein the nucleic acid encodes at least one polypeptide having a phospholipase activity, and the sequence identities are determined by analysis with a sequence comparison algorithm or by a visual inspection.

2. The isolated or recombinant nucleic acid of claim 1, wherein the sequence identity is at least about 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63% or 64%.

3. The isolated or recombinant nucleic acid of claim 1, wherein the sequence identity is at least about 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more, or is 100% sequence identity to SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:41, SEQ ID NO:43, SEQ ID NO:45, SEQ ID NO:47, SEQ ID NO:49, SEQ ID NO:51, SEQ ID NO:53, SEQ ID NO:55, SEQ ID NO:57, SEQ ID NO:59, SEQ ID NO:61, SEQ ID NO:63, SEQ ID NO:65, SEQ ID NO:67, SEQ ID NO:69, SEQ ID NO:71, SEQ ID NO:73, SEQ ID NO:75, SEQ ID NO:77, SEQ ID NO:79, SEQ ID NO:81, SEQ ID NO:83, SEQ ID NO:85, SEQ ID NO:87, SEQ ID

NO:89, SEQ ID NO:91, SEQ ID NO:93, SEQ ID NO:95, SEQ ID NO:97, SEQ ID NO:99,  
SEQ ID NO:101, SEQ ID NO:103, SEQ ID NO:105.

4. The isolated or recombinant nucleic acid of claim 1, wherein the  
5 sequence identity is over a region of at least about 50, 75, 100, 150, 200, 250, 300, 350, 400,  
450, 500, 550, 600, 650, 700, 750, 800, 850, 900, 950, 1000, 1050, 1100, 1150 or more  
residues, or the full length of a gene or a transcript.

5. The isolated or recombinant nucleic acid of claim 1, wherein the  
10 nucleic acid sequence comprises a sequence as set forth in SEQ ID NO:1, SEQ ID NO:3,  
SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID  
NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25,  
SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:35, SEQ ID  
NO:37, SEQ ID NO:39, SEQ ID NO:41, SEQ ID NO:43, SEQ ID NO:45, SEQ ID NO:47,  
15 SEQ ID NO:49, SEQ ID NO:51, SEQ ID NO:53, SEQ ID NO:55, SEQ ID NO:57, SEQ ID  
NO:59, SEQ ID NO:61, SEQ ID NO:63, SEQ ID NO:65, SEQ ID NO:67, SEQ ID NO:69,  
SEQ ID NO:71, SEQ ID NO:73, SEQ ID NO:75, SEQ ID NO:77, SEQ ID NO:79, SEQ ID  
NO:81, SEQ ID NO:83, SEQ ID NO:85, SEQ ID NO:87, SEQ ID NO:89, SEQ ID NO:91,  
SEQ ID NO:93, SEQ ID NO:95, SEQ ID NO:97, SEQ ID NO:99, SEQ ID NO:101, SEQ ID  
20 NO:103, SEQ ID NO:105.

6. The isolated or recombinant nucleic acid of claim 1, wherein the  
nucleic acid sequence encodes a polypeptide having a sequence as set forth in SEQ ID NO:2,  
SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID  
25 NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24,  
SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:34, SEQ ID  
NO:36, SEQ ID NO:38, SEQ ID NO:40, SEQ ID NO:42, SEQ ID NO:44, SEQ ID NO:46,  
SEQ ID NO:48, SEQ ID NO:50, SEQ ID NO:52, SEQ ID NO:54, SEQ ID NO:56, SEQ ID  
NO:58, SEQ ID NO:60, SEQ ID NO:62, SEQ ID NO:64, SEQ ID NO:66, SEQ ID NO:68,  
30 SEQ ID NO:70, SEQ ID NO:72, SEQ ID NO:74, SEQ ID NO:76, SEQ ID NO:78, SEQ ID  
NO:80, SEQ ID NO:82, SEQ ID NO:84, SEQ ID NO:86, SEQ ID NO:88, SEQ ID NO:90,  
SEQ ID NO:92, SEQ ID NO:94, SEQ ID NO:96, SEQ ID NO:98, SEQ ID NO:100, SEQ ID  
NO:102, SEQ ID NO:104, SEQ ID NO:106.

7. The isolated or recombinant nucleic acid of claim 1, wherein the sequence comparison algorithm is a BLAST version 2.2.2 algorithm where a filtering setting is set to blastall -p blastp -d "nr pataa" -F F, and all other options are set to default.

5 8. The isolated or recombinant nucleic acid of claim 1, wherein the phospholipase activity comprises catalyzing hydrolysis of a glycerolphosphate ester linkage.

9. The isolated or recombinant nucleic acid of claim 8, wherein the phospholipase activity comprises catalyzing hydrolysis of an ester linkage in a phospholipid  
10 in a vegetable oil.

10. The isolated or recombinant nucleic acid of claim 8, wherein the vegetable oil phospholipid comprises an oilseed phospholipid.

15 11. The isolated or recombinant nucleic acid of claim 1, wherein the phospholipase activity comprises a phospholipase C (PLC) activity.

12. The isolated or recombinant nucleic acid of claim 1, wherein the phospholipase activity comprises a phospholipase A (PLA) activity.

20 13. The isolated or recombinant nucleic acid of claim 1, wherein the phospholipase activity comprises a phospholipase B (PLB) activity.

25 14. The isolated or recombinant nucleic acid of claim 1, wherein the phospholipase activity comprises a phospholipase D (PLD) activity.

15. The isolated or recombinant nucleic acid of claim 1, wherein the phospholipase D activity comprises a phospholipase D1 or a phospholipase D2 activity.

30 16. The isolated or recombinant nucleic acid of claim 1, wherein the phospholipase activity comprises hydrolysis of a glycoprotein.

17. The isolated or recombinant nucleic acid of claim 16, wherein the glycoprotein comprises a potato tuber.

18. The isolated or recombinant nucleic acid of claim 1, wherein the phospholipase activity comprises a patatin enzymatic activity.

5 19. The isolated or recombinant nucleic acid of claim 18, wherein the phospholipase activity comprises a lipid acyl hydrolase (LAH) activity.

20. The isolated or recombinant nucleic acid of claim 1, wherein the phospholipase activity is thermostable.

10 21. The isolated or recombinant nucleic acid of claim 20, wherein the polypeptide retains a phospholipase activity under conditions comprising a temperature range of between about 37°C to about 95°C, or between about 55°C to about 85°C, or between about 70°C to about 75°C, or between about 70°C to about 95°C, or between about 90°C to about  
15 95°C.

22. The isolated or recombinant nucleic acid of claim 1, wherein the phospholipase activity is thermotolerant.

20 23. The isolated or recombinant nucleic acid of claim 22, wherein the polypeptide retains a phospholipase activity after exposure to a temperature in the range from greater than 37°C to about 95°C, from greater than 55°C to about 85°C, or between about 70°C to about 75°C, or from greater than 90°C to about 95°C.

25 24. An isolated or recombinant nucleic acid, wherein the nucleic acid comprises a sequence that hybridizes under stringent conditions to a nucleic acid comprising  
SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11,  
SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID  
NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:31, SEQ ID NO:33,  
30 SEQ ID NO:35, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:41, SEQ ID NO:43, SEQ ID  
NO:45, SEQ ID NO:47, SEQ ID NO:49, SEQ ID NO:51, SEQ ID NO:53, SEQ ID NO:55,  
SEQ ID NO:57, SEQ ID NO:59, SEQ ID NO:61, SEQ ID NO:63, SEQ ID NO:65, SEQ ID  
NO:67, SEQ ID NO:69, SEQ ID NO:71, SEQ ID NO:73, SEQ ID NO:75, SEQ ID NO:77,  
SEQ ID NO:79, SEQ ID NO:81, SEQ ID NO:83, SEQ ID NO:85, SEQ ID NO:87, SEQ ID

NO:89, SEQ ID NO:91, SEQ ID NO:93, SEQ ID NO:95, SEQ ID NO:97, SEQ ID NO:99, SEQ ID NO:101, SEQ ID NO:103, SEQ ID NO:105, wherein the nucleic acid encodes a polypeptide having a phospholipase activity.

5                   25.     The isolated or recombinant nucleic acid of claim 24, wherein the nucleic acid is at least about 50, 75, 100, 150, 200, 300, 400, 500, 600, 700, 800, 900, 1000 or more residues in length or the full length of the gene or transcript.

10                   26.     The isolated or recombinant nucleic acid of claim 24, wherein the stringent conditions include a wash step comprising a wash in 0.2X SSC at a temperature of about 65°C for about 15 minutes.

15                   27.     A nucleic acid probe for identifying a nucleic acid encoding a polypeptide with a phospholipase activity, wherein the probe comprises at least 10 consecutive bases of a sequence comprising SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:41, SEQ ID NO:43, SEQ ID NO:45, SEQ ID NO:47, SEQ ID NO:49, 20     SEQ ID NO:51, SEQ ID NO:53, SEQ ID NO:55, SEQ ID NO:57, SEQ ID NO:59, SEQ ID NO:61, SEQ ID NO:63, SEQ ID NO:65, SEQ ID NO:67, SEQ ID NO:69, SEQ ID NO:71, SEQ ID NO:73, SEQ ID NO:75, SEQ ID NO:77, SEQ ID NO:79, SEQ ID NO:81, SEQ ID NO:83, SEQ ID NO:85, SEQ ID NO:87, SEQ ID NO:89, SEQ ID NO:91, SEQ ID NO:93, SEQ ID NO:95, SEQ ID NO:97, SEQ ID NO:99, SEQ ID NO:101, SEQ ID NO:103, SEQ ID 25     NO:105, wherein the probe identifies the nucleic acid by binding or hybridization.

                  28.     The nucleic acid probe of claim 27, wherein the probe comprises an oligonucleotide comprising at least about 10 to 50, about 20 to 60, about 30 to 70, about 40 to 80, about 60 to 100, or about 50 to 150 consecutive bases.

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                  29.     A nucleic acid probe for identifying a nucleic acid encoding a polypeptide having a phospholipase activity, wherein the probe comprises a nucleic acid comprising at least about 10 consecutive residues of SEQ ID NO:1; SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ

ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:41, SEQ ID NO:43, SEQ ID NO:45, SEQ ID NO:47, SEQ ID NO:49, SEQ ID NO:51, SEQ ID NO:53, SEQ ID NO:55, SEQ ID NO:57, SEQ ID NO:59, SEQ ID NO:61, SEQ ID NO:63, SEQ ID NO:65, SEQ ID NO:67, SEQ ID NO:69, SEQ ID NO:71, SEQ ID NO:73, SEQ ID NO:75, SEQ ID NO:77, SEQ ID NO:79, SEQ ID NO:81, SEQ ID NO:83, SEQ ID NO:85, SEQ ID NO:87, SEQ ID NO:89, SEQ ID NO:91, SEQ ID NO:93, SEQ ID NO:95, SEQ ID NO:97, SEQ ID NO:99, SEQ ID NO:101, SEQ ID NO:103, SEQ ID NO:105, wherein the sequence identities are determined by analysis with a sequence comparison algorithm or by visual inspection.

30. The nucleic acid probe of claim 29, wherein the probe comprises an oligonucleotide comprising at least about 10 to 50, about 20 to 60, about 30 to 70, about 40 to 80, about 60 to 100, or about 50 to 150 consecutive bases.

31. An amplification primer sequence pair for amplifying a nucleic acid encoding a polypeptide having a phospholipase activity, wherein the primer pair is capable of amplifying a nucleic acid comprising a sequence as set forth in claim 1 or claim 24, or a subsequence thereof.

32. The amplification primer pair of claim 29, wherein a member of the amplification primer sequence pair comprises an oligonucleotide comprising at least about 10 to 50 consecutive bases, or about 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25 consecutive bases of the sequence.

33. An amplification primer pair, wherein the primer pair comprises a first member having a sequence as set forth by about the first (the 5') 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30 or more residues of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:41, SEQ ID NO:43, SEQ ID NO:45, SEQ ID NO:47, SEQ ID NO:49, SEQ ID NO:51, SEQ ID NO:53, SEQ ID NO:55, SEQ ID NO:57, SEQ ID NO:59, SEQ ID NO:61, SEQ ID NO:63, SEQ ID NO:65, SEQ ID NO:67, SEQ ID NO:69,

SEQ ID NO:71, SEQ ID NO:73, SEQ ID NO:75, SEQ ID NO:77, SEQ ID NO:79, SEQ ID NO:81, SEQ ID NO:83, SEQ ID NO:85, SEQ ID NO:87, SEQ ID NO:89, SEQ ID NO:91, SEQ ID NO:93, SEQ ID NO:95, SEQ ID NO:97, SEQ ID NO:99, SEQ ID NO:101, SEQ ID NO:103, SEQ ID NO:105, and a second member having a sequence as set forth by about the first (the 5') 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29 or 30 or more residues of the complementary strand of the first member.

34. A phospholipase-encoding nucleic acid generated by amplification of a polynucleotide using an amplification primer pair as set forth in claim 33.

35. The phospholipase-encoding nucleic acid of claim 34, wherein the amplification is by polymerase chain reaction (PCR).

36. The phospholipase-encoding nucleic acid of claim 34, wherein the nucleic acid generated by amplification of a gene library.

37. The phospholipase-encoding nucleic acid of claim 34, wherein the gene library is an environmental library.

38. An isolated or recombinant phospholipase encoded by a phospholipase-encoding nucleic acid as set forth in claim 34.

39. A method of amplifying a nucleic acid encoding a polypeptide having a phospholipase activity comprising amplification of a template nucleic acid with an amplification primer sequence pair capable of amplifying a nucleic acid sequence as set forth in claim 1 or claim 24, or a subsequence thereof.

40. A method for making a phospholipase comprising amplification of a nucleic acid with an amplification primer pair as set forth in claim 33 and expression of the amplified nucleic acid.

41. An expression cassette comprising a nucleic acid comprising a sequence as set forth in claim 1 or claim 24.

42. A vector comprising a nucleic acid comprising a sequence as set forth in claim 1 or claim 24.

5 43. A cloning vehicle comprising a nucleic acid comprising a sequence as set forth in claim 1 or claim 24, wherein the cloning vehicle comprises a viral vector, a plasmid, a phage, a phagemid, a cosmid, a fosmid, a bacteriophage or an artificial chromosome.

10 44. The cloning vehicle of claim 43, wherein the viral vector comprises an adenovirus vector, a retroviral vector or an adeno-associated viral vector.

45. The cloning vehicle of claim 43, comprising a bacterial artificial chromosome (BAC), a plasmid, a bacteriophage P1-derived vector (PAC), a yeast artificial chromosome (YAC), or a mammalian artificial chromosome (MAC).

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46. A transformed cell comprising a nucleic acid comprising a sequence as set forth in claim 1 or claim 24.

20 47. A transformed cell comprising an expression cassette as set forth in claim 41.

48. The transformed cell of claim 47, wherein the cell is a bacterial cell, a mammalian cell, a fungal cell, a yeast cell, an insect cell or a plant cell.

25 49. A transgenic non-human animal comprising a sequence as set forth in claim 1 or claim 24.

50. The transgenic non-human animal of claim 49, wherein the animal is a mouse.

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51. A transgenic plant comprising a sequence as set forth in claim 1 or claim 24.

52. The transgenic plant of claim 51, wherein the plant is a corn plant, a sorghum plant, a potato plant, a tomato plant, a wheat plant, an oilseed plant, a rapeseed plant, a soybean plant, a rice plant, a barley plant, a grass, a cottonseed, a palm, a sesame plant, a peanut plant, a sunflower plant or a tobacco plant.

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53. A transgenic seed comprising a sequence as set forth in claim 1 or claim 24.

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54. The transgenic seed of claim 53, wherein the seed is a corn seed, a wheat kernel, an oilseed, a rapeseed, a soybean seed, a palm kernel, a sunflower seed, a sesame seed, a rice, a barley, a peanut, a cottonseed, a palm, a peanut, a sesame seed, a sunflower seed or a tobacco plant seed.

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55. An antisense oligonucleotide comprising a nucleic acid sequence complementary to or capable of hybridizing under stringent conditions to a sequence as set forth in claim 1 or claim 24, or a subsequence thereof.

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56. The antisense oligonucleotide of claim 55, wherein the antisense oligonucleotide is between about 10 to 50, about 20 to 60, about 30 to 70, about 40 to 80, or about 60 to 100 bases in length.

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57. A method of inhibiting the translation of a phospholipase message in a cell comprising administering to the cell or expressing in the cell an antisense oligonucleotide comprising a nucleic acid sequence complementary to or capable of hybridizing under stringent conditions to a sequence as set forth in claim 1 or claim 24.

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58. A double-stranded inhibitory RNA (RNAi) molecule comprising a subsequence of a sequence as set forth in claim 1 or claim 24.

59. The double-stranded inhibitory RNA (RNAi) molecule of claim 58, wherein the RNAi is about 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25 or more duplex nucleotides in length.

60. A method of inhibiting the expression of a phospholipase in a cell comprising administering to the cell or expressing in the cell a double-stranded inhibitory RNA (iRNA), wherein the RNA comprises a subsequence of a sequence as set forth in claim 1 or claim 24.

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61. An isolated or recombinant polypeptide (i) having at least 50% sequence identity to SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:38, SEQ ID NO:40, SEQ ID NO:42, SEQ ID NO:44, SEQ ID NO:46, SEQ ID NO:48, SEQ ID NO:50, SEQ ID NO:52, SEQ ID NO:54, SEQ ID NO:56, SEQ ID NO:58, SEQ ID NO:60, SEQ ID NO:62, SEQ ID NO:64, SEQ ID NO:66, SEQ ID NO:68, SEQ ID NO:70, SEQ ID NO:72, SEQ ID NO:74, SEQ ID NO:76, SEQ ID NO:78, SEQ ID NO:80, SEQ ID NO:82, SEQ ID NO:84, SEQ ID NO:86, SEQ ID NO:88, SEQ ID NO:90, SEQ ID NO:92, SEQ ID NO:94, SEQ ID NO:96, SEQ ID NO:98, SEQ ID NO:100, SEQ ID NO:102, SEQ ID NO:104, SEQ ID NO:106, over a region of at least about 100 residues, wherein the sequence identities are determined by analysis with a sequence comparison algorithm or by a visual inspection, or, (ii) encoded by a nucleic acid having at least 50% sequence identity to a sequence as set forth in SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:41, SEQ ID NO:43, SEQ ID NO:45, SEQ ID NO:47, SEQ ID NO:49, SEQ ID NO:51, SEQ ID NO:53, SEQ ID NO:55, SEQ ID NO:57, SEQ ID NO:59, SEQ ID NO:61, SEQ ID NO:63, SEQ ID NO:65, SEQ ID NO:67, SEQ ID NO:69, SEQ ID NO:71, SEQ ID NO:73, SEQ ID NO:75, SEQ ID NO:77, SEQ ID NO:79, SEQ ID NO:81, SEQ ID NO:83, SEQ ID NO:85, SEQ ID NO:87, SEQ ID NO:89, SEQ ID NO:91, SEQ ID NO:93, SEQ ID NO:95, SEQ ID NO:97, SEQ ID NO:99, SEQ ID NO:101, SEQ ID NO:103, SEQ ID NO:105 over a region of at least about 100 residues, and the sequence identities are determined by analysis with a sequence comparison algorithm or by a visual inspection, or encoded by a nucleic acid capable of hybridizing under stringent conditions to a sequence as set forth in SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29,

SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:41, SEQ ID NO:43, SEQ ID NO:45, SEQ ID NO:47, SEQ ID NO:49, SEQ ID NO:51, SEQ ID NO:53, SEQ ID NO:55, SEQ ID NO:57, SEQ ID NO:59, SEQ ID NO:61, SEQ ID NO:63, SEQ ID NO:65, SEQ ID NO:67, SEQ ID NO:69, SEQ ID NO:71, SEQ ID NO:73, SEQ ID NO:75, SEQ ID NO:77, SEQ ID NO:79, SEQ ID NO:81, SEQ ID NO:83, SEQ ID NO:85, SEQ ID NO:87, SEQ ID NO:89, SEQ ID NO:91, SEQ ID NO:93, SEQ ID NO:95, SEQ ID NO:97, SEQ ID NO:99, SEQ ID NO:101, SEQ ID NO:103, SEQ ID NO:105.

62. The isolated or recombinant polypeptide of claim 61, wherein the sequence identity is over a region of at least about 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more, or is 100% sequence identity.

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63. The isolated or recombinant polypeptide of claim 61, wherein the sequence identity is over a region of at least about 10, 15, 20, 25, 30, 35, 40, 45, 50, 75, 100, 150, 200, 250, 300, 350, 400, 450, 500, 550, 600, 650, 700, 750, 800, 850, 900, 950, 1000, 1050 or more residues, or the full length of an enzyme.

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64. The isolated or recombinant polypeptide of claim 61, wherein the polypeptide has a sequence as set forth in SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:38, SEQ ID NO:40, SEQ ID NO:42, SEQ ID NO:44, SEQ ID NO:46, SEQ ID NO:48, SEQ ID NO:50, SEQ ID NO:52, SEQ ID NO:54, SEQ ID NO:56, SEQ ID NO:58, SEQ ID NO:60, SEQ ID NO:62, SEQ ID NO:64, SEQ ID NO:66, SEQ ID NO:68, SEQ ID NO:70, SEQ ID NO:72, SEQ ID NO:74, SEQ ID NO:76, SEQ ID NO:78, SEQ ID NO:80, SEQ ID NO:82, SEQ ID NO:84, SEQ ID NO:86, SEQ ID NO:88, SEQ ID NO:90, SEQ ID NO:92, SEQ ID NO:94, SEQ ID NO:96, SEQ ID NO:98, SEQ ID NO:100, SEQ ID NO:102, SEQ ID NO:104, SEQ ID NO:106.

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65. The isolated or recombinant polypeptide of claim 61, wherein the polypeptide has a phospholipase activity.

5 66. The isolated or recombinant polypeptide of claim 65, wherein the phospholipase activity comprises catalyzing hydrolysis of a glycerolphosphate ester linkage.

67. The isolated or recombinant polypeptide of claim 66, wherein the phospholipase activity comprises catalyzing hydrolysis of an ester linkage in a phospholipid in a vegetable oil.

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68. The isolated or recombinant polypeptide of claim 67, wherein the vegetable oil phospholipid comprises an oilseed phospholipid.

15 69. The isolated or recombinant polypeptide of claim 67, wherein the vegetable oil phospholipid is derived from a plant oil, a high phosphorous oil, a soy oil, a canola oil, a palm oil, a cottonseed oil, a corn oil, a palm kernel-derived phospholipid, a coconut oil, a peanut oil, a sesame oil, a fish oil, an algae phospholipid, a sunflower oil, an essential oil, a fruit seed oil, a grapeseed phospholipid, an apricot phospholipid, or a borage phospholipid.

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70. The isolated or recombinant polypeptide of claim 65, wherein the phospholipase activity comprises a phospholipase C (PLC) activity.

25 71. The isolated or recombinant polypeptide of claim 65, wherein the phospholipase activity comprises a phospholipase A (PLA) activity.

72. The isolated or recombinant polypeptide of claim 65, wherein the phospholipase activity comprises a phospholipase A1 or phospholipase A2 activity.

30 73. The isolated or recombinant polypeptide of claim 65, wherein the phospholipase activity comprises a phospholipase D (PLD) activity.

74. The isolated or recombinant polypeptide of claim 65, wherein the phospholipase D activity comprises a phospholipase D1 or a phospholipase D2 activity.

75. The isolated or recombinant polypeptide of claim 65, wherein the phospholipase activity comprises hydrolysis of a glycoprotein.

5 76. The isolated or recombinant polypeptide of claim 68, wherein the glycoprotein comprises a potato tuber.

77. The isolated or recombinant polypeptide of claim 65, wherein the phospholipase activity comprises a patatin enzymatic activity.

10 78. The isolated or recombinant polypeptide of claim 65, wherein the phospholipase activity comprises a lipid acyl hydrolase (LAH) activity.

15 79. The isolated or recombinant polypeptide of claim 65, wherein the phospholipase activity is thermostable.

80. The isolated or recombinant polypeptide of claim 79, wherein the polypeptide retains a phospholipase activity under conditions comprising a temperature range of between about 37°C to about 95°C, between about 55°C to about 85°C, between about  
20 70°C to about 95°C, between about 70°C to about 75°C, or between about 90°C to about 95°C.

81. The isolated or recombinant polypeptide of claim 65, wherein the phospholipase activity is thermotolerant.

25 82. The isolated or recombinant polypeptide of claim 81, wherein the polypeptide retains a phospholipase activity after exposure to a temperature in the range from greater than 37°C to about 95°C, from greater than 55°C to about 85°C, between about 70°C to about 75°C, or from greater than 90°C to about 95°C.

30 83. An isolated or recombinant polypeptide comprising a polypeptide as set forth in claim 61 and lacking a signal sequence.

84. An isolated or recombinant polypeptide comprising a polypeptide as set forth in claim 61 and having a heterologous signal sequence.

85. The isolated or recombinant polypeptide of claim 65, wherein the phospholipase activity comprises a specific activity at about 37°C in the range from about 100 to about 1000 units per milligram of protein, from about 500 to about 750 units per milligram of protein, from about 500 to about 1200 units per milligram of protein, or from about 750 to about 1000 units per milligram of protein.

86. The isolated or recombinant polypeptide of claim 81, wherein the thermotolerance comprises retention of at least half of the specific activity of the phospholipase at 37°C after being heated to an elevated temperature.

87. The isolated or recombinant polypeptide of claim 81, wherein the thermotolerance comprises retention of specific activity at 37°C in the range from about 500 to about 1200 units per milligram of protein after being heated to an elevated temperature.

88. The isolated or recombinant polypeptide of claim 61, wherein the polypeptide comprises at least one glycosylation site.

89. The isolated or recombinant polypeptide of claim 88, wherein the glycosylation is an N-linked glycosylation.

90. The isolated or recombinant polypeptide of claim 89, wherein the polypeptide is glycosylated after being expressed in an *P. pastoris* or an *S. pombe*.

91. The isolated or recombinant polypeptide of claim 65, wherein the polypeptide retains a phospholipase activity under conditions comprising about pH 6.5, pH 6.0, pH 5.5, 5.0, pH 4.5 or 4.0.

92. The isolated or recombinant polypeptide of claim 65, wherein the polypeptide retains a phospholipase activity under conditions comprising about pH 7.5, pH 8.0, pH 8.5, pH 9, pH 9.5, pH 10 or pH 10.5.

93. A protein preparation comprising a polypeptide as set forth in claim 61, wherein the protein preparation comprises a liquid, a solid or a gel.

94. A heterodimer comprising a polypeptide as set forth in claim 61 and a second domain.

5 95. The heterodimer of claim 94, wherein the second domain is a polypeptide and the heterodimer is a fusion protein.

96. The heterodimer of claim 94, wherein the second domain is an epitope or a tag.

10 97. A homodimer comprising a polypeptide as set forth in claim 61.

98. An immobilized polypeptide, wherein the polypeptide comprises a sequence as set forth in claim 61, or a subsequence thereof.

15 99. The immobilized polypeptide of claim 98, wherein the polypeptide is immobilized on a cell, a metal, a resin, a polymer, a ceramic, a glass, a microelectrode, a graphitic particle, a bead, a gel, a plate, an array or a capillary tube.

20 100. An array comprising an immobilized polypeptide as set forth in claim 61.

101. An array comprising an immobilized nucleic acid as set forth in claim 1 or claim 24.

25 102. An isolated or recombinant antibody that specifically binds to a polypeptide as set forth in claim 61.

30 103. The isolated or recombinant antibody of claim 102, wherein the antibody is a monoclonal or a polyclonal antibody.

104. A hybridoma comprising an antibody that specifically binds to a polypeptide as set forth in claim 61.

105. A method of isolating or identifying a polypeptide with a phospholipase activity comprising the steps of:

- (a) providing an antibody as set forth in claim 102;
- (b) providing a sample comprising polypeptides; and

5 (c) contacting the sample of step (b) with the antibody of step (a) under conditions wherein the antibody can specifically bind to the polypeptide, thereby isolating or identifying a polypeptide having a phospholipase activity.

106. A method of making an anti-phospholipase antibody comprising  
10 administering to a non-human animal a nucleic acid as set forth in claim 1 or claim 24 or a subsequence thereof in an amount sufficient to generate a humoral immune response, thereby making an anti-phospholipase antibody.

107. A method of making an anti-phospholipase antibody comprising  
15 administering to a non-human animal a polypeptide as set forth in claim 61 or a subsequence thereof in an amount sufficient to generate a humoral immune response, thereby making an anti-phospholipase antibody.

108. A method of producing a recombinant polypeptide comprising the  
20 steps of: (a) providing a nucleic acid operably linked to a promoter, wherein the nucleic acid comprises a sequence as set forth in claim 1 or claim 24; and (b) expressing the nucleic acid of step (a) under conditions that allow expression of the polypeptide, thereby producing a recombinant polypeptide.

25 109. The method of claim 108, further comprising transforming a host cell with the nucleic acid of step (a) followed by expressing the nucleic acid of step (a), thereby producing a recombinant polypeptide in a transformed cell.

110. A method for identifying a polypeptide having a phospholipase activity  
30 comprising the following steps:

- (a) providing a polypeptide as set forth in claim 65;
- (b) providing a phospholipase substrate; and
- (c) contacting the polypeptide with the substrate of step (b) and detecting a decrease in the amount of substrate or an increase in the amount of a reaction product,

wherein a decrease in the amount of the substrate or an increase in the amount of the reaction product detects a polypeptide having a phospholipase activity.

111. A method for identifying a phospholipase substrate comprising the following steps:

5 (a) providing a polypeptide as set forth in claim 65;

(b) providing a test substrate; and

(c) contacting the polypeptide of step (a) with the test substrate of step (b) and detecting a decrease in the amount of substrate or an increase in the amount of reaction

10 product, wherein a decrease in the amount of the substrate or an increase in the amount of a reaction product identifies the test substrate as a phospholipase substrate.

112. A method of determining whether a test compound specifically binds to a polypeptide comprising the following steps:

15 (a) expressing a nucleic acid or a vector comprising the nucleic acid under conditions permissive for translation of the nucleic acid to a polypeptide, wherein the nucleic acid has a sequence as set forth in claim 1 or claim 24;

(b) providing a test compound;

(c) contacting the polypeptide with the test compound; and

20 (d) determining whether the test compound of step (b) specifically binds to the polypeptide.

113. A method of determining whether a test compound specifically binds to a polypeptide comprising the following steps:

25 (a) providing a polypeptide as set forth in claim 61;

(b) providing a test compound;

(c) contacting the polypeptide with the test compound; and

(d) determining whether the test compound of step (b) specifically binds to the polypeptide.

30 114. A method for identifying a modulator of a phospholipase activity comprising the following steps:

- (a) providing a polypeptide as set forth in claim 65;
- (b) providing a test compound;

(c) contacting the polypeptide of step (a) with the test compound of step (b) and measuring an activity of the phospholipase, wherein a change in the phospholipase activity measured in the presence of the test compound compared to the activity in the absence of the test compound provides a determination that the test compound modulates the phospholipase activity.

115. The method of claim 114, wherein the phospholipase activity is measured by providing a phospholipase substrate and detecting a decrease in the amount of the substrate or an increase in the amount of a reaction product, or, an increase in the amount of the substrate or a decrease in the amount of a reaction product.

116. The method of claim 115, wherein a decrease in the amount of the substrate or an increase in the amount of the reaction product with the test compound as compared to the amount of substrate or reaction product without the test compound identifies the test compound as an activator of phospholipase activity.

117. The method of claim 115, wherein an increase in the amount of the substrate or a decrease in the amount of the reaction product with the test compound as compared to the amount of substrate or reaction product without the test compound identifies the test compound as an inhibitor of phospholipase activity.

118. A computer system comprising a processor and a data storage device wherein said data storage device has stored thereon a polypeptide sequence or a nucleic acid sequence, wherein the polypeptide sequence comprises sequence as set forth in claim 61, a polypeptide encoded by a nucleic acid as set forth in claim 1 or claim 24.

119. The computer system of claim 118, further comprising a sequence comparison algorithm and a data storage device having at least one reference sequence stored thereon.

120. The computer system of claim 119, wherein the sequence comparison algorithm comprises a computer program that indicates polymorphisms.

121. The computer system of claim 119, further comprising an identifier that identifies one or more features in said sequence.

122. A computer readable medium having stored thereon a polypeptide  
5 sequence or a nucleic acid sequence, wherein the polypeptide sequence comprises a polypeptide as set forth in claim 61; a polypeptide encoded by a nucleic acid as set forth in claim 1 or claim 24.

123. A method for identifying a feature in a sequence comprising the steps  
10 of: (a) reading the sequence using a computer program which identifies one or more features in a sequence, wherein the sequence comprises a polypeptide sequence or a nucleic acid sequence, wherein the polypeptide sequence comprises a polypeptide as set forth in claim 61; a polypeptide encoded by a nucleic acid as set forth in claim 1 or claim 24; and (b) identifying one or more features in the sequence with the computer program.

15

124. A method for comparing a first sequence to a second sequence comprising the steps of: (a) reading the first sequence and the second sequence through use of a computer program which compares sequences, wherein the first sequence comprises a polypeptide sequence or a nucleic acid sequence, wherein the polypeptide sequence  
20 comprises a polypeptide as set forth in claim 61 or a polypeptide encoded by a nucleic acid as set forth in claim 1 or claim 24; and (b) determining differences between the first sequence and the second sequence with the computer program.

125. The method of claim 124, wherein the step of determining differences  
25 between the first sequence and the second sequence further comprises the step of identifying polymorphisms.

126. The method of claim 124, further comprising an identifier that identifies one or more features in a sequence.

30

127. The method of claim 126, comprising reading the first sequence using a computer program and identifying one or more features in the sequence.

128. A method for isolating or recovering a nucleic acid encoding a polypeptide with a phospholipase activity from an environmental sample comprising the steps of:

- (a) providing an amplification primer sequence pair as set forth in claim 33;
- 5 (b) isolating a nucleic acid from the environmental sample or treating the environmental sample such that nucleic acid in the sample is accessible for hybridization to the amplification primer pair; and,
- 10 (c) combining the nucleic acid of step (b) with the amplification primer pair of step (a) and amplifying nucleic acid from the environmental sample, thereby isolating or recovering a nucleic acid encoding a polypeptide with a phospholipase activity from an environmental sample.

129. The method of claim 128, wherein each member of the amplification primer sequence pair comprises an oligonucleotide comprising at least about 10 to 50 consecutive bases of a sequence as set forth in SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:41, SEQ ID NO:43, SEQ ID NO:45, SEQ ID NO:47, SEQ ID NO:49, SEQ ID NO:51, SEQ ID NO:53, SEQ ID NO:55, SEQ ID NO:57, SEQ ID NO:59, SEQ ID NO:61, SEQ ID NO:63, SEQ ID NO:65, SEQ ID NO:67, SEQ ID NO:69, SEQ ID NO:71, SEQ ID NO:73, SEQ ID NO:75, SEQ ID NO:77, SEQ ID NO:79, SEQ ID NO:81, SEQ ID NO:83, SEQ ID NO:85, SEQ ID NO:87, SEQ ID NO:89, SEQ ID NO:91, SEQ ID NO:93, SEQ ID NO:95, SEQ ID NO:97, SEQ ID NO:99, SEQ ID NO:101, SEQ ID NO:103, SEQ ID NO:105, or a subsequence thereof.

130. A method for isolating or recovering a nucleic acid encoding a polypeptide with a phospholipase activity from an environmental sample comprising the steps of:

- 30 (a) providing a polynucleotide probe comprising a sequence as set forth in claim 1 or claim 24, or a subsequence thereof;
- (b) isolating a nucleic acid from the environmental sample or treating the environmental sample such that nucleic acid in the sample is accessible for hybridization to a polynucleotide probe of step (a);

(c) combining the isolated nucleic acid or the treated environmental sample of step (b) with the polynucleotide probe of step (a); and

(d) isolating a nucleic acid that specifically hybridizes with the polynucleotide probe of step (a), thereby isolating or recovering a nucleic acid encoding a polypeptide with a phospholipase activity from an environmental sample.

131. The method of claim 128 or claim 130, wherein the environmental sample comprises a water sample, a liquid sample, a soil sample, an air sample or a biological sample.

132. The method of claim 131, wherein the biological sample is derived from a bacterial cell, a protozoan cell, an insect cell, a yeast cell, a plant cell, a fungal cell or a mammalian cell.

133. A method of generating a variant of a nucleic acid encoding a polypeptide with a phospholipase activity comprising the steps of:

(a) providing a template nucleic acid comprising a sequence as set forth in claim 1 or claim 24; and

(b) modifying, deleting or adding one or more nucleotides in the template sequence, or a combination thereof, to generate a variant of the template nucleic acid.

134. The method of claim 133, further comprising expressing the variant nucleic acid to generate a variant phospholipase polypeptide.

135. The method of claim 133, wherein the modifications, additions or deletions are introduced by a method comprising error-prone PCR, shuffling, oligonucleotide-directed mutagenesis, assembly PCR, sexual PCR mutagenesis, in vivo mutagenesis, cassette mutagenesis, recursive ensemble mutagenesis, exponential ensemble mutagenesis, site-specific mutagenesis, gene reassembly, gene site saturated mutagenesis (GSSM), synthetic ligation reassembly (SLR) and a combination thereof.

136. The method of claim 133, wherein the modifications, additions or deletions are introduced by a method comprising recombination, recursive sequence recombination, phosphothioate-modified DNA mutagenesis, uracil-containing template

mutagenesis, gapped duplex mutagenesis, point mismatch repair mutagenesis, repair-deficient host strain mutagenesis, chemical mutagenesis, radiogenic mutagenesis, deletion mutagenesis, restriction-selection mutagenesis, restriction-purification mutagenesis, artificial gene synthesis, ensemble mutagenesis, chimeric nucleic acid multimer creation and a  
5 combination thereof.

137. The method of claim 133, wherein the method is iteratively repeated until a phospholipase having an altered or different activity or an altered or different stability from that of a polypeptide encoded by the template nucleic acid is produced.

10 138. The method of claim 137, wherein the variant phospholipase polypeptide is thermotolerant, and retains some activity after being exposed to an elevated temperature.

15 139. The method of claim 137, wherein the variant phospholipase polypeptide has increased glycosylation as compared to the phospholipase encoded by a template nucleic acid.

20 140. The method of claim 137, wherein the variant phospholipase polypeptide has a phospholipase activity under a high temperature, wherein the phospholipase encoded by the template nucleic acid is not active under the high temperature.

25 141. The method of claim 133, wherein the method is iteratively repeated until a phospholipase coding sequence having an altered codon usage from that of the template nucleic acid is produced.

30 142. The method of claim 133, wherein the method is iteratively repeated until a phospholipase gene having higher or lower level of message expression or stability from that of the template nucleic acid is produced.

143. A method for modifying codons in a nucleic acid encoding a polypeptide with a phospholipase activity to increase its expression in a host cell, the method comprising the following steps:

(a) providing a nucleic acid encoding a polypeptide with a phospholipase activity comprising a sequence as set forth in claim 1 or claim 24; and,

5 (b) identifying a non-preferred or a less preferred codon in the nucleic acid of step (a) and replacing it with a preferred or neutrally used codon encoding the same amino acid as the replaced codon, wherein a preferred codon is a codon over-represented in coding sequences in genes in the host cell and a non-preferred or less preferred codon is a codon under-represented in coding sequences in genes in the host cell, thereby modifying the nucleic acid to increase its expression in a host cell.

10 144. A method for modifying codons in a nucleic acid encoding a phospholipase polypeptide, the method comprising the following steps:

(a) providing a nucleic acid encoding a polypeptide with a phospholipase activity comprising a sequence as set forth in claim 1 or claim 24; and,

15 (b) identifying a codon in the nucleic acid of step (a) and replacing it with a different codon encoding the same amino acid as the replaced codon, thereby modifying codons in a nucleic acid encoding a phospholipase.

20 145. A method for modifying codons in a nucleic acid encoding a phospholipase polypeptide to increase its expression in a host cell, the method comprising the following steps:

(a) providing a nucleic acid encoding a phospholipase polypeptide comprising a sequence as set forth in claim 1 or claim 24; and,

25 (b) identifying a non-preferred or a less preferred codon in the nucleic acid of step (a) and replacing it with a preferred or neutrally used codon encoding the same amino acid as the replaced codon, wherein a preferred codon is a codon over-represented in coding sequences in genes in the host cell and a non-preferred or less preferred codon is a codon under-represented in coding sequences in genes in the host cell, thereby modifying the nucleic acid to increase its expression in a host cell.

30 146. A method for modifying a codon in a nucleic acid encoding a polypeptide having a phospholipase activity to decrease its expression in a host cell, the method comprising the following steps:

(a) providing a nucleic acid encoding a phospholipase polypeptide comprising a sequence as set forth in claim 1 or claim 24; and

(b) identifying at least one preferred codon in the nucleic acid of step (a) and replacing it with a non-preferred or less preferred codon encoding the same amino acid as the replaced codon, wherein a preferred codon is a codon over-represented in coding sequences in genes in a host cell and a non-preferred or less preferred codon is a codon under-

5 represented in coding sequences in genes in the host cell, thereby modifying the nucleic acid to decrease its expression in a host cell.

147. The method of claim 146, wherein the host cell is a bacterial cell, a fungal cell, an insect cell, a yeast cell, a plant cell or a mammalian cell.

10

148. A method for producing a library of nucleic acids encoding a plurality of modified phospholipase active sites or substrate binding sites, wherein the modified active sites or substrate binding sites are derived from a first nucleic acid comprising a sequence encoding a first active site or a first substrate binding site the method comprising the

15 following steps:

(a) providing a first nucleic acid encoding a first active site or first substrate binding site, wherein the first nucleic acid sequence comprises a sequence that hybridizes under stringent conditions to a sequence as set forth in SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:41, SEQ ID NO:43, SEQ ID NO:45, SEQ ID NO:47, SEQ ID NO:49, SEQ ID NO:51, SEQ ID NO:53, SEQ ID NO:55, SEQ ID NO:57, SEQ ID NO:59, SEQ ID NO:61, SEQ ID NO:63, SEQ ID NO:65, SEQ ID NO:67, SEQ ID NO:69, SEQ ID NO:71, SEQ ID NO:73, SEQ ID NO:75, SEQ ID NO:77, SEQ ID NO:79, SEQ ID NO:81, SEQ ID NO:83, SEQ ID NO:85, SEQ ID NO:87, SEQ ID NO:89, SEQ ID NO:91, SEQ ID NO:93, SEQ ID NO:95, SEQ ID NO:97, SEQ ID NO:99, SEQ ID NO:101, SEQ ID NO:103, SEQ ID NO:105, or a subsequence thereof, and the nucleic acid encodes a phospholipase active site or a phospholipase substrate binding site;

30

(b) providing a set of mutagenic oligonucleotides that encode naturally-occurring amino acid variants at a plurality of targeted codons in the first nucleic acid; and,

(c) using the set of mutagenic oligonucleotides to generate a set of active site-encoding or substrate binding site-encoding variant nucleic acids encoding a range of amino acid variations at each amino acid codon that was mutagenized, thereby producing a library

of nucleic acids encoding a plurality of modified phospholipase active sites or substrate binding sites.

149. The method of claim 148, comprising mutagenizing the first nucleic acid of step (a) by a method comprising an optimized directed evolution system, gene site-saturation mutagenesis (GSSM), or a synthetic ligation reassembly (SLR).

150. The method of claim 148, comprising mutagenizing the first nucleic acid of step (a) or variants by a method comprising error-prone PCR, shuffling, oligonucleotide-directed mutagenesis, assembly PCR, sexual PCR mutagenesis, in vivo mutagenesis, cassette mutagenesis, recursive ensemble mutagenesis, exponential ensemble mutagenesis, site-specific mutagenesis, gene reassembly, gene site saturated mutagenesis (GSSM), synthetic ligation reassembly (SLR) and a combination thereof.

151. The method of claim 148, comprising mutagenizing the first nucleic acid of step (a) or variants by a method comprising recombination, recursive sequence recombination, phosphothioate-modified DNA mutagenesis, uracil-containing template mutagenesis, gapped duplex mutagenesis, point mismatch repair mutagenesis, repair-deficient host strain mutagenesis, chemical mutagenesis, radiogenic mutagenesis, deletion mutagenesis, restriction-selection mutagenesis, restriction-purification mutagenesis, artificial gene synthesis, ensemble mutagenesis, chimeric nucleic acid multimer creation and a combination thereof.

152. A method for making a small molecule comprising the following steps:  
(a) providing a plurality of biosynthetic enzymes capable of synthesizing or modifying a small molecule, wherein one of the enzymes comprises a phospholipase enzyme encoded by a nucleic acid comprising a sequence as set forth in claim 1 or claim 24;  
(b) providing a substrate for at least one of the enzymes of step (a); and  
(c) reacting the substrate of step (b) with the enzymes under conditions that facilitate a plurality of biocatalytic reactions to generate a small molecule by a series of biocatalytic reactions.

153. A method for modifying a small molecule comprising the following steps:

(a) providing a phospholipase enzyme, wherein the enzyme comprises a polypeptide as set forth in claim 65, or a polypeptide encoded by a nucleic acid comprising a nucleic acid sequence as set forth in claim 1 or claim 24;

(b) providing a small molecule; and

5 (c) reacting the enzyme of step (a) with the small molecule of step (b) under conditions that facilitate an enzymatic reaction catalyzed by the phospholipase enzyme, thereby modifying a small molecule by a phospholipase enzymatic reaction.

10 154. The method of claim 153, comprising a plurality of small molecule substrates for the enzyme of step (a), thereby generating a library of modified small molecules produced by at least one enzymatic reaction catalyzed by the phospholipase enzyme.

15 155. The method of claim 153, further comprising a plurality of additional enzymes under conditions that facilitate a plurality of biocatalytic reactions by the enzymes to form a library of modified small molecules produced by the plurality of enzymatic reactions.

20 156. The method of claim 155, further comprising the step of testing the library to determine if a particular modified small molecule which exhibits a desired activity is present within the library.

25 157. The method of claim 156, wherein the step of testing the library further comprises the steps of systematically eliminating all but one of the biocatalytic reactions used to produce a portion of the plurality of the modified small molecules within the library by testing the portion of the modified small molecule for the presence or absence of the particular modified small molecule with a desired activity, and identifying at least one specific biocatalytic reaction that produces the particular modified small molecule of desired activity.

30

158. A method for determining a functional fragment of a phospholipase enzyme comprising the steps of:

(a) providing a phospholipase enzyme, wherein the enzyme comprises a polypeptide as set forth in claim 65, or a polypeptide encoded by a nucleic acid as set forth in claim 1 or claim 24; and

5 (b) deleting a plurality of amino acid residues from the sequence of step (a) and testing the remaining subsequence for a phospholipase activity, thereby determining a functional fragment of a phospholipase enzyme.

10 159. The method of claim 158, wherein the phospholipase activity is measured by providing a phospholipase substrate and detecting a decrease in the amount of the substrate or an increase in the amount of a reaction product.

160. A method for whole cell engineering of new or modified phenotypes by using real-time metabolic flux analysis, the method comprising the following steps:

15 (a) making a modified cell by modifying the genetic composition of a cell, wherein the genetic composition is modified by addition to the cell of a nucleic acid comprising a sequence as set forth in claim 1 or claim 24;

(b) culturing the modified cell to generate a plurality of modified cells;

(c) measuring at least one metabolic parameter of the cell by monitoring the cell culture of step (b) in real time; and,

20 (d) analyzing the data of step (c) to determine if the measured parameter differs from a comparable measurement in an unmodified cell under similar conditions, thereby identifying an engineered phenotype in the cell using real-time metabolic flux analysis.

25 161. The method of claim 160, wherein the genetic composition of the cell is modified by a method comprising deletion of a sequence or modification of a sequence in the cell, or, knocking out the expression of a gene.

30 162. The method of claim 160, further comprising selecting a cell comprising a newly engineered phenotype.

163. The method of claim 162, further comprising culturing the selected cell, thereby generating a new cell strain comprising a newly engineered phenotype.

164. An isolated or recombinant signal sequence consisting of a sequence as set forth in residues 1 to 16, 1 to 17, 1 to 18, 1 to 19, 1 to 20, 1 to 21, 1 to 22, 1 to 23, 1 to 24, 1 to 25, 1 to 26, 1 to 27, 1 to 28, 1 to 28, 1 to 30 or 1 to 31, 1 to 32 or 1 to 33 of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:38, SEQ ID NO:40, SEQ ID NO:42, SEQ ID NO:44, SEQ ID NO:46, SEQ ID NO:48, SEQ ID NO:50, SEQ ID NO:52, SEQ ID NO:54, SEQ ID NO:56, SEQ ID NO:58, SEQ ID NO:60, SEQ ID NO:62, SEQ ID NO:64, SEQ ID NO:66, SEQ ID NO:68, SEQ ID NO:70, SEQ ID NO:72, SEQ ID NO:74, SEQ ID NO:76, SEQ ID NO:78, SEQ ID NO:80, SEQ ID NO:82, SEQ ID NO:84, SEQ ID NO:86, SEQ ID NO:88, SEQ ID NO:90, SEQ ID NO:92, SEQ ID NO:94, SEQ ID NO:96, SEQ ID NO:98, SEQ ID NO:100, SEQ ID NO:102, SEQ ID NO:104, SEQ ID NO:106.

165. A chimeric polypeptide comprising at least a first domain comprising signal peptide (SP) having a sequence as set forth in claim 164, and at least a second domain comprising a heterologous polypeptide or peptide, wherein the heterologous polypeptide or peptide is not naturally associated with the signal peptide (SP).

166. The chimeric polypeptide of claim 165, wherein the heterologous polypeptide or peptide is not a phospholipase.

167. The chimeric polypeptide of claim 165, wherein the heterologous polypeptide or peptide is amino terminal to, carboxy terminal to or on both ends of the signal peptide (SP) or a catalytic domain (CD).

168. An isolated or recombinant nucleic acid encoding a chimeric polypeptide, wherein the chimeric polypeptide comprises at least a first domain comprising signal peptide (SP) having a sequence as set forth in claim 164 and at least a second domain comprising a heterologous polypeptide or peptide, wherein the heterologous polypeptide or peptide is not naturally associated with the signal peptide (SP).

169. A method of increasing thermotolerance or thermostability of a phospholipase polypeptide, the method comprising glycosylating a phospholipase, wherein

the polypeptide comprises at least thirty contiguous amino acids of a polypeptide as set forth in claim 61, or a polypeptide encoded by a nucleic acid as set forth in claim 1 or claim 24, thereby increasing the thermotolerance or thermostability of the phospholipase.

5           170. A method for overexpressing a recombinant phospholipase in a cell comprising expressing a vector comprising a nucleic acid sequence as set forth in claim 1 or claim 24, wherein overexpression is effected by use of a high activity promoter, a dicistronic vector or by gene amplification of the vector.

10           171. A method of making a transgenic plant comprising the following steps:  
          (a) introducing a heterologous nucleic acid sequence into the cell, wherein the heterologous nucleic sequence comprises a sequence as set forth in claim 1 or claim 24, thereby producing a transformed plant cell;  
          (b) producing a transgenic plant from the transformed cell.

15           172. The method as set forth in claim 171, wherein the step (a) further comprises introducing the heterologous nucleic acid sequence by electroporation or microinjection of plant cell protoplasts.

20           173. The method as set forth in claim 171, wherein the step (a) comprises introducing the heterologous nucleic acid sequence directly to plant tissue by DNA particle bombardment or by using an *Agrobacterium tumefaciens* host.

25           174. A method of expressing a heterologous nucleic acid sequence in a plant cell comprising the following steps:

          (a) transforming the plant cell with a heterologous nucleic acid sequence operably linked to a promoter, wherein the heterologous nucleic sequence comprises a sequence as set forth in claim 1 or claim 24;

30           (b) growing the plant under conditions wherein the heterologous nucleic acids sequence is expressed in the plant cell.

          175. A method for hydrolyzing, breaking up or disrupting a phospholipid-comprising composition comprising the following steps:

(a) providing a polypeptide having a phospholipase activity as set forth in claim 65, or a polypeptide encoded by a nucleic acid as set forth in claim 1 or claim 24;

(b) providing a composition comprising a phospholipid; and

5 (c) contacting the polypeptide of step (a) with the composition of step (b) under conditions wherein the phospholipase hydrolyzes, breaks up or disrupts the phospholipid-comprising composition.

176. The method as set forth in claim 175, wherein the composition comprises a phospholipid-comprising lipid bilayer or membrane.

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177. The method as set forth in claim 175, wherein the composition comprises a plant cell, a bacterial cell, a yeast cell, an insect cell, or an animal cell.

15

178. A method for liquefying or removing a phospholipid-comprising composition comprising the following steps:

(a) providing a polypeptide having a phospholipase activity as set forth in claim 65, or a polypeptide encoded by a nucleic acid as set forth in claim 1 or claim 24;

(b) providing a composition comprising a phospholipid; and

20 (c) contacting the polypeptide of step (a) with the composition of step (b) under conditions wherein the phospholipase removes or liquefies the phospholipid-comprising composition.

25

179. A detergent composition comprising a polypeptide as set forth in claim 65, or a polypeptide encoded by a nucleic acid as set forth in claim 1 or claim 24, wherein the polypeptide has a phospholipase activity.

180. The detergent composition of claim 179, wherein the phospholipase is a nonsurface-active phospholipase or a surface-active phospholipase.

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181. The detergent composition of claim 179, wherein the phospholipase is formulated in a non-aqueous liquid composition, a cast solid, a granular form, a particulate form, a compressed tablet, a gel form, a paste or a slurry form.

182. A method for washing an object comprising the following steps:

(a) providing a composition comprising a polypeptide having a phospholipase activity as set forth in claim 65, or a polypeptide encoded by a nucleic acid as set forth in claim 1 or claim 24;

(b) providing an object; and

5 (c) contacting the polypeptide of step (a) and the object of step (b) under conditions wherein the composition can wash the object.

183. A method for degumming an oil comprising the following steps:

10 (a) providing a composition comprising a polypeptide having a phospholipase activity as set forth in claim 65, or a polypeptide encoded by a nucleic acid as set forth in claim 1 or claim 24;

(b) providing an composition comprising an phospholipid-containing fat or oil; and

15 (c) contacting the polypeptide of step (a) and the composition of step (b) under conditions wherein the polypeptide can catalyze the hydrolysis of a phospholipid in the composition.

184. The method of claim 183, wherein the oil-comprising composition comprises a plant, an animal, an algae or a fish oil or fat.

20 185. The method of claim 184, wherein plant oil comprises a soybean oil, a rapeseed oil, a corn oil, an oil from a palm kernel, a canola oil, a sunflower oil, a sesame oil or a peanut oil.

25 186. The method of claim 183, wherein the polypeptide hydrolyzes a phosphatide from a hydratable and/or a non-hydratable phospholipid in the oil-comprising composition.

30 187. The method of claim 183, wherein the polypeptide hydrolyzes a phosphatide at a glyceryl phosphoester bond to generate a diglyceride and water-soluble phosphate compound.

188. The method of claim 183, wherein the polypeptide has a phospholipase C activity.

189. The method of claim 183, wherein the polypeptide has a phospholipase D activity and a phosphatase enzyme is also added.

5 190. The method of claim 183, wherein the contacting comprises hydrolysis of a hydrated phospholipid in an oil.

191. The method of claim 183, wherein the hydrolysis conditions of step (c) comprise a temperature of about 20°C to 40°C at an alkaline pH.

10 192. The method of claim 190, wherein the alkaline conditions comprise a pH of about pH 8 to pH 10.

193. The method of claim 183, wherein the hydrolysis conditions of step (c) comprise a reaction time of about 3 to 10 minutes.

194. The method of claim 183, wherein the hydrolysis conditions of step (c) comprise hydrolysis of hydratable and non-hydratable phospholipids in oil at a temperature of about 50°C to 60°C, at a pH of about pH 5 to pH 6.5 using a reaction time of about 30 to 60 minutes.

195. The method of claim 183, wherein the polypeptide is bound to a filter and the phospholipid-containing fat or oil is passed through the filter.

25 196. The method of claim 183, wherein the polypeptide is added to a solution comprising the phospholipid-containing fat or oil and then the solution is passed through a filter.

197. A method for converting a non-hydratable phospholipid to a hydratable form comprising the following steps:

30 (a) providing a composition comprising a polypeptide having a phospholipase activity as set forth in claim 65, or a polypeptide encoded by a nucleic acid as set forth in claim 1 or claim 24;

(b) providing an composition comprising a non-hydratable phospholipid; and

(c) contacting the polypeptide of step (a) and the composition of step (b) under conditions wherein the polypeptide converts the non-hydratable phospholipid to a hydratable form.

5                   198.    The method of claim 197, wherein the polypeptide has a phospholipase C activity.

                  199.    The method of claim 197, wherein the polypeptide has a phospholipase D activity and a phosphatase enzyme is also added.

10                   200.    A method for caustic refining of a phospholipid-containing composition comprising the following steps:

                  (a) providing a composition comprising a polypeptide having a phospholipase activity as set forth in claim 65, or a polypeptide encoded by a nucleic acid as set forth in  
15   claim 1 or claim 24;

                  (b) providing an composition comprising a phospholipid; and

                  (c) contacting the polypeptide of step (a) with the composition of step (b) before, during or after the caustic refining.

20                   201.    The method of claim 200, wherein the polypeptide has a phospholipase C activity.

                  202.    The method of claim 200, wherein the polypeptide having a phospholipase activity is added before caustic refining and the composition comprising the  
25   phospholipid comprises a plant and the polypeptide is expressed transgenically in the plant, the polypeptide having a phospholipase activity added during crushing of a seed or other plant part, or, the polypeptide having a phospholipase activity added following crushing or prior to refining.

30                   203.    The method of claim 200, wherein the polypeptide having a phospholipase activity is added during caustic refining and varying levels of acid and caustic are added depending on levels of phosphorous and levels of free fatty acids.

204. The method of claim 200, wherein the polypeptide having a phospholipase activity is added after caustic refining; in an intense mixer or retention mixer prior to separation; following a heating step; in a centrifuge; in a soapstock; in a washwater; or, during bleaching or deodorizing steps.

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205. A method for purification of a phytosterol or a triterpene comprising the following steps:

(a) providing a composition comprising a polypeptide having a phospholipase activity as set forth in claim 65, or a polypeptide encoded by a nucleic acid as set forth in claim 1 or claim 24;

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(b) providing an composition comprising a phytosterol or a triterpene; and

(c) contacting the polypeptide of step (a) with the composition of step (b) under conditions wherein the polypeptide can catalyze the hydrolysis of a phospholipid in the composition.

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206. The method of claim 205, wherein the polypeptide has a phospholipase C activity.

207. The method of claim 205, wherein the phytosterol or a triterpene

20 comprises a plant sterol.

208. The method of claim 207, wherein the plant sterol is derived from a vegetable oil.

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209. The method of claim 208, wherein the vegetable oil comprises a coconut oil, canola oil, cocoa butter oil, corn oil, cottonseed oil, linseed oil, olive oil, palm oil, peanut oil, oil derived from a rice bran, safflower oil, sesame oil, soybean oil or a sunflower oil.

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210. The method of claim 205, further comprising use of nonpolar solvents to quantitatively extract free phytosterols and phytosteryl fatty-acid esters.

211. The method of claim 205, wherein the phytosterol or a triterpene comprises a  $\beta$ -sitosterol, a campesterol, a stigmasterol, a stigmastanol, a  $\beta$ -sitostanol, a sitostanol, a desmosterol, a chalinasterol, a poriferasterol, a clionasterol or a brassicasterol.

5 212. A method for refining a crude oil comprising the following steps:

(a) providing a composition comprising a polypeptide having a phospholipase activity as set forth in claim 65, or a polypeptide encoded by a nucleic acid as set forth in claim 1 or claim 24;

(b) providing a composition comprising an oil comprising a phospholipid; and

10 (c) contacting the polypeptide of step (a) with the composition of step (b) under conditions wherein the polypeptide can catalyze the hydrolysis of a phospholipid in the composition.

213. The method of claim 212, wherein the polypeptide has a phospholipase

15 C activity.

214. The method of claim 212, wherein the polypeptide having a phospholipase activity is in a water solution that is added to the composition.

20 215. The method of claim 214, wherein the water level is between about 0.5 to 5%.

216. The method of claim 214, wherein the process time is less than about 2 hours.

25 217. The method of claim 216, wherein the process time is less than about 60 minutes.

218. The method of claim 217, wherein the process time is less than about 30 minutes, less than about 15 minutes, or less than about 5 minutes.

219. The method of claim 212, wherein the hydrolysis conditions comprise a temperature of between about 25°C-70°C.

220. The method of claim 212, wherein the hydrolysis conditions comprise use of caustics.

221. The method of claim 212, wherein the hydrolysis conditions comprise a pH of between about pH 3 and pH 10.

222. The method of claim 212, wherein the hydrolysis conditions comprise addition of emulsifiers and/or mixing after the contacting of step (c).

223. The method of claim 212, comprising addition of an emulsion-breaker and/or heat to promote separation of an aqueous phase.

224. The method of claim 212, comprising degumming before the contacting step to collect lecithin by centrifugation and then adding a PLC, a PLC and/or a PLA to remove non-hydratable phospholipids.

225. The method of claim 212, comprising water degumming of crude oil to less than 10 ppm for edible oils and subsequent physical refining to less than about 50 ppm for biodiesel oils.

226. The method of claim 212, comprising addition of acid to promote hydration of non-hydratable phospholipids.

227. A method for degumming an oil or a fat comprising the following steps:

(a) providing a composition comprising a polypeptide having a phospholipase activity as set forth in claim 65, or a polypeptide encoded by a nucleic acid as set forth in claim 1 or claim 24, wherein the phospholipase activity comprises a phospholipase D activity, and a phosphatase enzyme;

(b) providing an composition comprising an phospholipid-containing fat or oil; and

(c) contacting the polypeptide of step (a) and the composition of step (b) under conditions wherein the polypeptide can catalyze the hydrolysis of a phospholipid in the composition.

228. A composition having the equivalent of a phospholipase C activity comprising providing a composition comprising a polypeptide having a phospholipase activity as set forth in claim 65, or a polypeptide encoded by a nucleic acid as set forth in claim 1 or claim 24, wherein the phospholipase activity comprises a phospholipase D activity, and a phosphatase enzyme.